

## CLAIMS

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1. A method of selecting a probe for a target nucleic acid sequence, the method comprising the steps of:
  - a) hybridizing three or more candidate probes with a first composition comprising the target nucleic acid sequence;
  - b) determining a first hybridization signal for each candidate probe;
  - c) hybridizing the three or more candidate probes with a second composition comprising the target nucleic acid sequence;
  - d) determining a second hybridization signal for each candidate probe;
  - e) calculating a hybridization signal ratio of the first hybridization signal to the second hybridization signal for each candidate probe;
  - f) calculating an average hybridization signal ratio for the three or more candidate probes; and
  - g) selecting the candidate probe by comparing a candidate probe's hybridization signal ratio to the average hybridization signal ratio.
2. The method of claim 1, wherein the target nucleic acid comprises cDNA.
3. The method of claim 2, wherein the cDNA is derived from a mammalian cell.
4. The method of claim 3, wherein the mammalian cell is a rat cell.
5. The method of claim 3, wherein the mammalian cell is a human cell.
6. The method of claim 1, wherein the target nucleic acid comprises genomic DNA.
7. The method of claim 6, wherein the genomic DNA is derived from a mammalian cell.

8. The method of claim 7, wherein the mammalian cell is a rat cell.

9. The method of claim 7, wherein the mammalian cell is a human cell.

5 10. The method of claim 1, wherein the target nucleic acid comprises RNA.

11. The method of claim 10, wherein the RNA is derived from a mammalian cell.

12. The method of claim 11, wherein the mammalian cell is a rat cell.

10 13. The method of claim 11, wherein the mammalian cell is a human cell.

14. The method of claim 1, wherein the target nucleic acid is derived from a prokaryote.

15 15. The method of claim 1, wherein the target nucleic acid is derived from a virus.

16. The method of claim 1, wherein the three or more candidate probes comprise a nucleic acid sequence complementary to the target sequence.

20 17. The method of claim 1, wherein the three or more candidate probes comprise a nucleic acid sequence complementary to an expressed sequence or the expressed sequence's complement.

25 18. The method of claim 17, wherein the expressed sequence comprises a mammalian expressed sequence.

19. The method of claim 18, wherein the mammalian expressed sequence is a rat expressed sequence.

20. The method of claim 18, wherein the mammalian expressed sequence is a human expressed sequence.

5 21. The method of claim 1, wherein the three or more candidate probes comprise a nucleic acid sequence complementary to a genomic nucleic acid sequence.

10 22. The method of claim 1, wherein the three or more candidate probes comprise a nucleic acid sequence complementary to a viral nucleic acid sequence or the viral nucleic acid sequence's complement.

15 23. The method of claim 1, wherein the three or more candidate probes comprise a candidate probe comprising a nucleic acid sequence complementary to at least 15 contiguous nucleotides of the target sequence.

20 24. The method of claim 23, wherein each of the three or more candidate probes comprise a nucleic acid sequence complementary to at least 15 contiguous nucleotides of the target sequence.

25 25. The method of claim 1, wherein the three or more candidate probes comprise a candidate probe comprising a nucleic acid sequence complementary to at least 30 contiguous nucleotides of the target sequence.

26. The method of claim 25, wherein each of the three or more candidate probes comprise a nucleic acid sequence complementary to at least 30 contiguous nucleotides of the target sequence.

27. The method of claim 23, wherein the three or more candidate probes comprise a candidate probe comprising a nucleic acid sequence complementary to less than 100 contiguous nucleotides of the target sequence.

28. The method of claim 25, wherein the three or more candidate probes comprise a candidate probe comprising a nucleic acid sequence complementary to less than 100 contiguous nucleotides of the target sequence.

5        29. The method of claim 1, wherein a nucleic acid array comprises the three or more candidate probes.

10      30. The method of claim 1, wherein the first composition and the second composition comprise a concentration of the target sequence, the concentration within the first composition differing from the concentration within the second composition.

15      31. The method of claim 30, wherein the first composition is derived from a different tissue type from that in which the second composition is derived.

20      32. The method of claim 30, wherein the first composition and the second composition are derived from a cell type grown at growth conditions, the growth conditions from which the first composition is derived differing from the growth conditions from which the second composition is derived.

25      33. The method of claim 30, wherein the first composition and the second composition comprise different concentrations of a stock composition derived from one or more cells.

34. The method of claim 1, wherein the hybridizing comprises stringent conditions.

35. The method of claim 1, wherein the target nucleic acid comprises a detectable moiety.

36. The method of claim 1, wherein the target nucleic acid comprises a first partner of a binding pair.

37. The method of claim 36, wherein a second partner of the binding pair comprises a label.

38. The method of claim 36, wherein the first partner comprises biotin.

39. The method of claim 37, wherein the second partner comprises biotin.

40. The method of claim 1, wherein determining a first hybridization signal comprises averaging more than one hybridization signal for the candidate probe hybridized with the first composition.

41. The method of claim 1, wherein determining a second hybridization signal comprises averaging more than one hybridization signal for the candidate probe hybridized with the second composition.

42. The method of claim 1, further comprising the steps of:

20 c1) hybridizing the three or more candidate probes with a third composition comprising the target nucleic acid sequence;

d1) determining a third hybridization signal for each candidate probe;

e1) calculating a second hybridization signal ratio of the first hybridization signal to the third hybridization signal for each candidate probe;

f1) calculating an average second hybridization signal ratio for the three or more candidate probes; and

25 g1) selecting the candidate probe by comparing a candidate probe's second hybridization signal ratio to the average second hybridization signal ratio

43. The method of claim 42, wherein the selecting comprises selecting the candidate probe by comparing the candidate probe's hybridization signal ratio and second hybridization signal ratio to the average hybridization signal ratio and average second hybridization signal ratio.

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44. The method of claim 42, further comprising the steps of:

- e2) calculating a third hybridization signal ratio of the second hybridization signal to the third hybridization signal for each candidate probe; and
- f2) calculating an average third hybridization signal ratio for the three or more candidate probes.

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45. The method of claim 44, wherein the selecting comprises selecting the candidate probe by comparing the candidate probe's hybridization signal ratio, second hybridization signal ratio, and third hybridization signal ratio to the average hybridization signal ratio, average second hybridization signal ratio, and average third hybridization signal ratio.

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46. The method of claim 1, wherein selecting comprises selecting the candidate probe having a hybridization signal ratio closest to the average hybridization signal ratio.

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47. The method of claim 42, wherein selecting comprises selecting the candidate probe having a second hybridization signal ratio closest to the average second hybridization signal ratio.

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48. The method of claim 43, wherein the selecting comprises selecting the candidate probe having a hybridization signal ratio and second hybridization signal ratio closest to the average hybridization signal ratio and average second hybridization signal ratio.

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5                   49. The method of claim 45, wherein the selecting comprises selecting the candidate probe having a hybridization signal ratio, second hybridization signal ratio, and third hybridization signal ratio closest to the average hybridization signal ratio, average second hybridization signal ratio, and average third hybridization signal ratio.

10                  50. The method of claim 1, wherein the first composition comprises a first concentration of the target nucleic acid sequence and the second composition comprises a second concentration of the target nucleic acid sequence, the method comprising:  
alternatively to step f), a step of calculating a concentration ratio of the first concentration of the target nucleic acid to the second concentration of the target nucleic acid; and  
alternatively to step g), selecting the candidate probe by comparing the candidate probe's hybridization signal ratio to the concentration ratio.

15                  51. The method of claim 66, wherein the selecting comprises selecting the candidate probe having a hybridization signal ratio closest to the concentration ratio.

20                  52. A method of making an oligonucleotide array, comprising the steps of:  
a) hybridizing three or more candidate probes comprising a nucleic acid sequence with a first composition comprising the target nucleic acid sequence;  
b) determining a first hybridization signal for each candidate probe;  
c) hybridizing the three or more candidate probes with a second composition comprising the target nucleic acid sequence;  
d) determining a second hybridization signal for each candidate probe;

5                   e) calculating a hybridization signal ratio of the first hybridization signal to the second hybridization signal for each candidate probe;

f) calculating an average hybridization signal ratio for the three or more candidate probes;

g) selecting the candidate probe by comparing the candidate probe's hybridization signal ratio to the average hybridization signal ratio, yielding a first probe; and

h) constructing an oligonucleotide array comprising a probe comprising the nucleic acid sequence of the first probe.

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53. The method of claim 52, wherein steps a) through g) are repeated with a second target sequence and second candidate probes to yield a second probe and constructing a nucleic acid array comprising the first probe and the second probe.

15                   54. The method of claim 52, wherein selecting comprises selecting the candidate probe having a hybridization signal ratio closest to the average hybridization signal ratio.

20                   55. An oligonucleotide array comprising at least 10 probes to 10 different human genes, the probes selected using the method of claim 1.

56. The oligonucleotide array of claim 55 comprising at least 100 probes to 100 different human genes, the probes selected using the method of claim 1.

25                   57. The oligonucleotide array of claim 56 comprising at least 1000 probes to 1000 different human genes, the probes selected using the method of claim 1.

58. The oligonucleotide array of claim 57 comprising at least 5000 probes to 5000 different human genes, the probes selected using the method of claim 1.

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59. The oligonucleotide array of claim 58 comprising at least 10000 probes to 10000 different human genes, the probes selected using the method of claim 1.

60. The oligonucleotide array of claim 55, wherein every probe of the array represents a different gene.

61. The oligonucleotide array of claim 58 wherein every probe of the array represents a different gene.

10 62. A method of analyzing the expression of a gene within a source, comprising:  
a) hybridizing a nucleic acid composition derived from the source with the oligonucleotide array of claim 47 comprising a probe representing the gene; and  
b) determining hybridization of a nucleic acid within the composition to the probe representing the gene, wherein hybridization of a nucleic acid within the composition to the probe representing the gene indicates expression of the gene within the source.

15 63. The method of claim 62, wherein the expression of at least 10 genes is analyzed.

20 64. The method of claim 63, wherein the expression of at least 100 genes is analyzed.

65. The method of claim 64, wherein the expression of at least 1000 genes is analyzed.

25 66. The method of claim 65, wherein the expression of at least 5000 genes is analyzed.

67. The method of claim 66, wherein the expression of at least 10000 genes is analyzed.